Frequency and causes of infectious abortion in a dairy herd in Queretaro, Mexico

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Abstract

The objective of this study was to determine the frequency of infectious bovine abortion and to identify some of its causes, specifically brucellosis, leptospirosis, bovine viral diarrhea, infectious bovine rhinotracheitis, and neosporosis. The study was carried out in a dairy herd in the state of Queretaro, Mexico, between September 2002 and March 2003. At the beginning of the study, blood samples were taken from a random 33% of the 300 lactating or pregnant cows; antibodies against *Leptospira interrogans* were the most commonly identified, in 91% of the 99 samples. Blood samples were also taken 14 to 28 d after the 26 subsequent abortions in the herd in the 6-mo study period, as well as from 22 cows that had not aborted within 5 d after the abortions in the other group. Seroconversion was most frequent for *L. hardjo*, occurring in 8 (67%) of the 12 dams that aborted after the initial serologic sampling and for which paired serum samples were therefore available. Of the 16 collected fetuses, 10 had histologic lesions suggesting infection in various organs, the features correlating with the serologic results for the dams in 7 cases. Thus, the abortions may have been caused by more than 1 infectious agent.

Résumé

Cette étude avait comme objectif de déterminer la fréquence des avortements infectieux chez les bovins et d'identifier quelques unes de ses causes, plus spécialement la brucellose, la leptospirose, la diarrhée virale bovine, la rhinotrachéite infectieuse bovine et la néosporose. Cette étude a été réalisée dans un troupeau de bovin laitier dans l'état de Queretaro, Mexique, durant la période entre septembre 2002 et mars 2003. Au début de l'étude des échantillons sanguins ont été prélevés de 33 % des 300 vaches en lactation ou gestantes sélectionnées de manière aléatoire; des anticorps dirigés contre Leptospira interrogans *étaient les plus fréquemment identifiés, dans 91 % des 99 échantillons. Des échantillons de sang ont également été prélevés 14 à 28 j après les 26 avortements subséquents dans le troupeau durant la période d'étude de 6 mois, ainsi que de 22 vaches qui n'avaient pas avorté dans les 5 j suivant les avortements dans l'autre groupe. Une séroconversion dirigée contre* L. hardjo *était la plus fréquente, étant rencontrée chez 8 (67 %) des 12 vaches ayant avortées après la première analyse sérologique et pour lesquelles des échantillons de sérum pairés étaient donc disponibles. Parmi les 16 fœtus amassés, 10 présentaient des lésions histologiques suggestives d'infection dans différents organes, les trouvailles étant corrélées avec les résultats sérologiques des mères en 7 occasions. Ainsi, les avortements peuvent avoir été causés par plus d'un agent infectieux.*

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Abortion among dairy cows, which occurs at 42 to 265 d of gestation, represents a major economic burden to the cattle industry. Although there are no official numbers, the estimated loss caused by abortion in California is about US$200 million per year (1). In large dairy herds, it has been suggested that an abortion rate of 5% to 10% is to be expected, and this estimate is used as a parameter (endemic abortion rate); greater percentages are termed abortion storms (2–4). Infectious agents have been identified in an estimated 20% to 40% of cases in which samples are submitted to diagnostic laboratories (2,4). Results of serologic testing for certain infectious agents in an animal that has aborted must be interpreted with great caution, as it is difficult to differentiate antibodies due to vaccination from those due to natural infection. The validity of serologic results increases when paired serum samples from several animals in the affected herd are examined. When the antibody titer increases 4-fold or more, or when animals free of antibody in the 1st sample manifest significant titers in the 2nd sample, it can be assumed that the infection is active in the herd (5).

In Mexico, little is known about the causes of infectious abortion in dairy herds. The purpose of the present study was to determine the frequency of infectious abortion in dairy cattle and to identify some of the main causes, such as brucellosis, leptospirosis, bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), and neosporosis. We used as an example a dairy herd in the state of Queretaro, one of the most important dairy areas in Mexico and one in which these diseases are included in official prevention and control programs and serologic tests are available.

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Table I. Seroconversion[a] among cows that aborted in Queretaro, Mexico, in 2002–2003, as determined from the titer or optical density of antibodies related to 5 diseases

Cow	BRU;	Optical density			*Leptospira interrogans* serovar; titer							
no.	titer	IBR	BVD	NEO	*betaviae*	*canicola*	*grippotyphosa*	*hardjo*	*icterohaemorrhagiae*	*pomona*	*tarasovi*	*wolffi*
1	0									1:50		1:50
	1:200									1:200		1:200
2	0							1:200				
	1:200							1:800				
3				0.03				0		1:50		
				0.60				1:100		1:200		
4								0		0	1:50	
								1:400		1:200	1:200	
5		0.063	0.003		0		0			1:50		
		0.480	0.350		1:800		1:800			1:800		
6				0.05		0	0	0	1:50			
				2.80		1:400	1:200	1:800	1:400			
7					0					0		
					1:800					1:100		
8					0	0		0				
					1:200	1:400		1:200				
9					0	0		0			1:400	
					1:800	1:100		1:800			1:800	
10			0.008		0	0		0				
			0.100		1:100	1:100		1:200				
11												0
												1:400
12					0	0		0				
					1:100	1:100		1:800				

BRU — brucellosis; IBR — infectious bovine rhinotracheitis; BVD — bovine viral diarrhea; NEO — neosporosis.

[a] Value for 2nd blood sample underneath value for 1st sample.

The study was carried out between September 2002 and March 2003 at a ranch on the Queretaro–Bernal Highway, in the municipality of Colon. The herd consisted of 260 lactating Holstein cows aged between 3 and 7 y and 40 pregnant heifers aged between 18 and 24 mo that calved during the study. The cows were housed in covered free-stall barns with concrete floors. They were milked twice a day and fed a combination of grain, hay, and silage. They had last been vaccinated in April 2002 against the causative agents of BVD, IBR, *Bovine parainfluenza virus 3* (bPIV3), bovine syncytial virus, and 5 serovars of *Leptospira interrogans* (*L. hardjo*, *L. icterohaemorrhagiae*, *L. pomona*, *L. canicola*, and *L. grippotyphosa*). In January 2002, RB51 vaccine against *Brucella abortus* had been administered. This vaccine does not interfere with the tests established by the Mexican Official Norm (NOM-041-ZOO-1995), National Campaign Against Animal Brucellosis in Mexico, Secretary of Agriculture, Cattle Ranching, Rural Development, Fishing and Feeding (SAGARPA). The cows had been revaccinated against brucellosis in January 2003, with complete doses of RB51 vaccine.

To determine the frequency of seropositivity for the different etiologic agents of abortion, we collected 10-mL blood samples from 99 cows or heifers by puncturing the coccygeal vein in September 2002. The size of the animal sample was established in conformity with the study budget, taking into account the cost of diagnosing the 5 diseases being studied. The make-up of the sample was defined by simple random selection and consisted of 33% of the lactating cows and pregnant heifers.

Brucellosis was identified with the use of a card agglutination test, and positive results were confirmed by means of the rivanol test, which has a sensitivity of 99.2% and a specificity of 55.4% (6), with the use of the reagents and the method described by the National Campaign Against Animal Brucellosis in Mexico (7); rivanol agglutination titers $\geq$ 1:50 are considered positive for brucellosis. Leptospirosis was identified by means of the microscopic agglutination test (MAT), which has a sensitivity of 98.2% and a specificity of 96.4% (8), with the use of the reagents and the method described by the Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México (9); agglutination titers of *Leptospira* antibodies $\geq$ 1:100 are considered positive for leptospirosis (9,10). Antibodies against *Neospora caninum* and the BVD and IBR viruses were assessed by indirect enzyme-linked immunosorbent assay (ELISA). For the neosporosis ELISA, we used the HerdChek kit (sensitivity 98.6%, specificity 98.9%), according to the instructions of the manufacturer (IDEXX Laboratories, Westbrook. Maine, USA); the presence or absence of antibody to *Neospora* is determined by the sample-to-positive (S/P) ratio for each sample. Serum samples with a ratio $\geq$ 0.50 were classified as positive. For the BVD ELISA, we used the CHEKIT-BVD-SERO II kit, which has a sensitivity of 91% and a specificity of 57% (11), according to the instructions of

Table II. Correlation, or lack of, between histologic lesions in 10 aborted fetuses and postabortion serologic results for their dams

		Type of lesion					
Fetus no.	Cow no.	Suppurative bronchopneumonia	Lymphocytic myocarditis	Hepatitis	Lymphocytic interstitial nephritis	Consistent with	Dam seropositivity
1	3		+	Lymphocytic periportal		NEO	NEO + LEP
2	1	+		Lymphocytic multifocal	+	BRU or LEP	BRU + LEP
3	5		+			NEO	IBR + BVD + LEP
4	10	With arteritis				BRU	BVD + LEP
5	6		+	Lymphocytic periportal	+	NEO, BRU, or LEP	NEO + LEP
6	4		+	Lymphocytic periportal		NEO	LEP
7	7	+		Granulomatous periportal with necrotic foci		BRU or LEP	LEP
8	2	With arteritis				BRU	BRU + LEP
9	8	+					
					+	BRU or LEP	LEP
10	12	+		Lymphocytic multifocal		BRU or LEP	LEP

LEP — leptospirosis.

the manufacturer (Bommeli Diagnostics, Intervet, Westbrook. Maine, USA); serum samples with an S/P ratio $> 40\%$ were classified as positive. For the IBR ELISA, we used the CHEKIT-Trachitest Serum kit (sensitivity $> 99\%$, specificity 99.6%), according to the the instructions of the manufacturer (Bommeli Diagnostics); serum samples with an S/P proportion $> 45\%$ were classified as positive.

For each cow in the herd that aborted during the study period, we collected and similarly tested a 10-mL blood sample within 14 to 28 d after the abortion. Of the 26 cows that aborted, only 25 could be evaluated. We also collected a blood sample from 25 cows in the herd that did not abort, within 5 d of the recording of an abortion in the other group (5). Antibody titers for each agent in the 2 groups of cows were analyzed by means of Student's *t* test.

Some of the cows in the initial group of 99 were re-evaluated when they subsequently aborted. In comparing their 2 blood samples, we considered leptospirosis, IBR, BVD, and neosporosis to be the probable cause of abortion if the antibody titer in the 2nd sample was 4 or more times the titer in the 1st sample (5,12), and brucellosis was indicated as the probable cause if the result of the rivanol test was positive.

Tours were made twice a day to collect fetuses aborted from cows previously identified as pregnant. Each fetus underwent necropsy. Samples approximately 0.5 cm in diameter were obtained from the different organs, fixed in 10% neutral buffered formalin, and embedded in paraffin; sections 3 μm wide were stained with hematoxylin and eosin for histologic study.

The data were analyzed with the use of descriptive statistics and the following epidemiologic measures: cumulative incidence (number of new abortions during the study period divided by the population risk) and true incidence (number of new abortions during the study period divided by the number of months at risk during the study period).

In the 1st set of 99 blood samples, antibodies against *Leptospira* and IBR virus were detected in 91% and 90%, respectively, antibodies against BVD virus and *N. caninum* in 70% and 64%, respectively, and antibodies against *Brucella* in 24%. The cumulative incidence of abortion during the 6-mo study period was 86 per 1000 cows. The true incidence was 13 per 1000 mo at risk. Most of the abortions occurred in October and February.

There were no statistically significant differences ($P > 0.05$) in the mean titer of antibodies to the IBR and BVD viruses and *N. caninum* between the cows that had aborted and those that had not, but the titers of antibody against *Brucella* and some serovars of *Leptospira* were higher in 6 and 15 cows, respectively, that had aborted than in the cows that had not aborted. Of the 25 cows that had aborted, 12 (48%) were among the 99 initially sampled, for a cumulative incidence of 121 per 1000 cows. Table I compares the antibody titers in these 12 cows before and after abortion. Seroconversion was most often observed for *Leptospira* serovars, especially *L. hardjo*, for which seroconversion occurred in 8 (67%) of the 12 cows; for brucellosis, BVD, and neosporosis, only 2 cows (17%) seroconverted, and for IBR only 1 cow (8%) did so.

The fetuses were obtained from 16 of the 26 (62%) abortions. The fetal age ranged from 45 d to 8 mo. Histologic lesions in lung, myocardium, liver, and kidney were found in only 10 (62%) of the 16 fetuses. In 7 cases, the lesions were consistent with those previously described for brucellosis, leptospirosis, and neosporosis, the diseases identified by the postabortion serologic testing of the dams (Table II).

Determining the causes of livestock abortion represents a great challenge. Some of the reasons for low diagnostic success are inadequate history or sampling, autolysis of the fetus or placenta, the fact that fetal membranes are frequently unavailable for examination, the fact that no serologic test completely differentiates antibodies detected in vaccinated animals and those found in infected animals, and the fact that more than 1 organism is often involved. In our study, it was not possible to examine the placentas, and some cows that had aborted were seropositive for more than 1 organism, complicating the interpretation of results. The rate of positivity for serovars of *L. interrogans* in the 1st sampling (91%) was higher than the rates reported by other researchers in Mexico (13,14): 31% and 63%, respectively, *L. hardjo* being the most frequent serovar,

as in our study. Even though the cows in our study had last been vaccinated in April 2002 against the *canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae*, and *pomona* serovars, low antibody titers would be expected by September. Others have stated that 12 wk after vaccination the titers usually decline below 1:100 (12,15). According to Mickelsen and Evermann (16), since leptospiral serovars do not induce a prolonged immune response, titers of 1:800 or above for a single serovar and without vaccination in the past 90 d suggests an active infection, which may have been the case in this herd. In the United States, *L. hardjo* genotype *hardjo-bovis* has been identified as the serovar most often associated with abortions (17). Two-thirds of the cows in our study that had aborted showed positivity for this serovar after abortion.

According to the results of the 1st serum sampling and the fetal lesions, it appears that *N. caninum* may be involved in abortions in cows. A seroprevalence study of bovine neosporosis in Mexico involving 50 dairy herds, some from Queretaro, revealed a high prevalence of *N. caninum* antibodies (72%) among cows that had been experiencing a high abortion rate (18). These findings suggest that many animals have been exposed to the agent. Since the herd that we studied had been vaccinated against IBR and BVD, which usually results in stable antibody titers, it may be significant that the titer increased in only 2 cows (1 for IBR and BVD and 1 for BVD) that had aborted. However, microscopic lesions consistent with viral infection were not found in the fetuses, suggesting that this was not an active viral infection, although this possibility cannot be completely discarded.

Our results also showed that most of cows that had aborted showed seroconversion related to at least 1 serovar of *Leptospira*, as might be expected, even though the 2nd sample was taken 14 to 28 d after the abortion. According to Kirkbride (12), MAT titers will be maximal at the time of abortion, although Yaeger and Holler (19) stated that *Leptospira* antibody titers are stable or already diminishing at the time of abortion because the active infection usually occurs previously.

Our attempt to correlate the serologic results in cows after abortion and the histopathologic findings in the fetuses suggested that the lesions were caused mainly by bacterial agents (*Leptospira* and *Brucella* spp.) followed by *Neospora* and, therefore, that there is a need to isolate the organisms from the fetal fluids or diseased tissues for an etiologic diagnosis. Owing to problems with the collection and transport of fetal tissues to the laboratory, we were unable to perform a bacteriologic study.

Thus, we can conclude that the abortions may have been caused by more than 1 infectious agent and that *L. interrogans* serovar *hardjo* may have been the agent most frequently involved. Our results support the theory that the diagnosis of abortion should be based on evaluation of the dam as well as the fetus.

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